

Title: The Effects of Iron Complexing Ligands on the Long Term Ecosystem Response to Iron Enrichment of HNLC waters

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Substantial increases in the concentrations of the stronger of two Fe(III) complexing organic ligand classes measured during the mesoscale Fe enrichment studies IronEx II and SOIREE appeared to sharply curtailed Fe availability to diatoms and thus limited the efficiency of carbon sequestration to the deep. Detailed observations during IronEx II (eastern equatorial Pacific) and SOIREE (Southern Ocean) indicate that the diatoms began re-experiencing Fe stress even though dissolved Fe concentrations remained elevated in the patch. This surprising outcome likely is related to the observed increased concentrations of strong Fe(III)-complexing ligands in seawater. Preliminary findings from other studies indicate that diatoms may not readily obtain Fe from these chemical species whereas Fe bound by strong ligands appears to support growth of cyanobacteria and nanoflagellates. The difficulty in assessing the likelihood of these changes with in-situ mesoscale experiments is the extended monitoring period needed to capture the long-term trajectory of the carbon cycle. A more detailed understanding of Fe complexing ligand effects on long-term ecosystem structure and carbon cycling is essential to ascertain not only the effect of Fe enrichment on short-term carbon sequestration in the oceans, but also the potential effect of Fe enrichment in modifying ecosystem structure.

The project addressed the following central hypothesis:

The complexation of Fe by stronger ligand classes in seawater will sharply curtail Fe availability to diatoms, result in a change in phytoplankton speciation, and thus limit the efficiency of carbon sequestration to deep ocean waters.

The specific goals of the proposed work are to employ novel manipulation experiments to: 1) determine how different natural and synthetic Fe chelators affect Fe availability to phytoplankton species that are representative of offshore HNLC waters, 2) elucidate how the changes in absolute concentrations of these chelators (such as observed during IronEx II and SOIREE) will affect the ecosystem response beyond the normal (1-4 week) period of observations, and 3) ascertain how changes in the ligand composition affect cell sinking and aggregation rates; measures of the efficiency of carbon sequestration to the deep.

Experimental Approach: Our primary emphasis was to use laboratory and field-based, semi-continuous batch cultures and continuous cultures to measure how a suite of natural ligand analogs as well as Fe-complexing ligands isolated from seawater affect Fe availability to phytoplankton. Growth rates, macronutrient utilization rates, and physiological parameters (variable fluorescence, cell size, Fe use efficiencies, and light dependent rates of carbon and Fe uptake) were used to ascertain the organism-specific responses to Fe supplied in these different chemical forms with and without elevated UV treatment. The relationship between ligand types and individual cell sinking rates and the kinetics of cell aggregation was determined to better assess the linkage between Fe ligands and export of carbon and macronutrients to the deep. The project combined laboratory experiments with field experiments on 3 separate extended research cruises. In the latter case, the novel sea-going continuous culture system was used to evaluate for

the first time the effect that different strong and weaker Fe complexing ligands had on community structure of the algal assemblage.

Central Finding: The experiments and field studies clearly supported the central hypothesis. The addition of strong Fe-complexing ligands at low (nanomolar) concentrations sharply curtailed the growth of eukaryotic phytoplankton and negatively affected the photophysiological parameters of the cell. During the second Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study (SEEDS II), eukaryotic phytoplankton (diatoms in particular) increased their biomass slightly upon enrichment of surface waters with iron, but the growth response of nanoplankton was very muted. Despite the presence of significantly elevated dissolved Fe concentrations within the patch waters (~0.5-1 nM), further iron amendments in deckboard incubation studies resulted in markedly increased growth rates, demonstrating that the nanoplankton remained Fe limited. This lack of growth response was attributed mainly to a persistent excess of strong Fe(III) complexing ligands (Takeda et al., unpublished data) regulating the chemical speciation, and thus biological availability, of dissolved iron in the patch. We also showed that this limitation could be partly reversed by small amendments of copper, which has been implicated as a key constituent for the high affinity iron uptake systems of yeast, which can access strongly bound Fe.

Our studies show that marine planktonic ecosystem respond to large inputs of iron by restricting the availability of dissolved Fe to large eukaryotic phytoplankton. In other words, the planktonic ecosystem works towards greatly restricting diatom growth. Aside then from the grand unknowns about the effect these enrichments will have on surface and deep ocean environments, our study indicates that the efficiency of carbon sequestration by iron enrichment of High Nitrate Low Chlorophyll surface waters of the oceans is very low.

Project Accomplishments

Equipment Fabrication and Methods Development: A significant portion of the work was directed to the fabrication of a novel sea-going continuous culture system for use in deckboard incubation experiments. To our knowledge, only one other system with this capability exists in the world. This successful system has been used in field experiments in coastal waters of the Pacific Northwest and in offshore waters of the subarctic Pacific. Additional fabrication and method developments included:

1. Several Plexiglas settling columns were fabricated, successfully field-tested and used experimentally in the Bering Sea and other environments above. Experiments both in the laboratory and on research cruises have generated valid results.
2. A couette device was designed and fabricated for used for measuring aggregation rates of phytoplankton under both laboratory and field conditions.
3. Image analysis software protocols were developed for automated microscopic analysis of particle numbers and sizes for use with the settling columns and Couette devices.
4. The Alcian blue method for analysis of transparent extracellular polymers (TEP) in seawater was validated on laboratory and field samples from the settling column and Couette device experiments.
5. A custom trace metal clean ultra-violet (UV) pretreatment system was acquired for use in photodegradation experiments of natural Fe-binding ligands in

seawater. This device was used during incubation experiments in the spring/summer of 2004 and 2006.

Research Accomplishments and Summary of Findings:

1. We participated in 6 cruises of opportunity to test the central and offshoot hypotheses developed during the project. These include cruises to the Bering Sea (1), the subarctic Pacific (2) and the coastal waters off the Washington/British Columbia coasts (3)). In each case, both continuous cultures, standard large-volume batch cultures, and small bottle batch culture experiments were conducted.
2. Combined with laboratory culture experiments, these monoclonal and natural population cultures tested the effects of different organic ligand structures, concentrations, and photochemical susceptibility on the comparative availability of Fe to large eukaryotic phytoplankton over picoplankton populations. The ligands chosen in these experiments were expected analogs of strong and weak Fe-binding ligand classes in seawater. Parameters measured to assess these effects included the growth response (Chlorophyll a, cell counts, photosynthetic efficiency, nutrient acquisition rates, Fe uptake) of eukaryotic and prokaryotic phytoplankton and heterotrophic bacteria.
3. We tested and utilized a range of cytoprobes for measuring cell specific intracellular Fe (Phen Green SK, Phen Green FL), photosynthetic efficiency (DCMU-induced oxidative stress measure with Rhodamine 123 and DCMU-induced cellular NO accumulation measured with DAF-FM, (4-amino-5-methylamino-2',7'-difluorescein)). The method for measuring cell-specific growth rates using the ratio of probe-detected DNA (SYBR Green) to cellular protein (Sytox) have been tested and finalized for a suite of organisms. Our experiments showed that measuring cell-specific Fe uptake by flow cytometry with Phen Green FL was unsuccessful due to lack the needed sensitivity.
4. The project has supported 3 graduate students and provided a major if not complete part of their thesis research.
5. The availability of Fe to eukaryotic phytoplankton decreases in the presence of strong Fe-binding ligands (siderophores), while Fe bound to the weaker type ligand (protoporphyrin IX) appears to be readily available.
6. We find that some eukaryotic phytoplankton can adapt to utilized strongly bound Fe over longer times scales (days).
7. The addition of Cu enhances the kinetics of this adaptation, signifying that Cu is involved in Fe acquisition from strong Fe-complexing ligands.
8. As predicted, there are taxon-specific differences in cell sinking rates (diatoms faster in general than other microplanktonn).
9. Nutrient stress generally increases net sinking rates for all phytoplankton, but Fe-limited cells sinking faster than N-limited cells.
10. Cells grown under Fe-stress conditions exhibit greater cell stickiness, and generate large aggregated particles faster under constant shear conditions than Fe-replete cells. This Fe-enhanced increase in particle formation may be the mechanism behind the higher sinking rates measured under Fe-stress than under N-stress.

Research Products:

Work from this project have been presented in the following international/national meetings

The Synergy Of Iron, Copper And The Toxicity Of Diatoms. Wells, M. L., Trick, C. G., and Hughes M. P. ASLO/TOS Ocean Research Conference. Honolulu, HI, February 2004

Iron Limitation And Copper Effects In The Juan De Fuca Eddy. Trick, C. G., Wells, M. L., Cochlan, W., Pickell, L., and McClintock, L. ASLO/TOS Ocean Research Conference. Honolulu, HI, February 2004

Iron limitation of natural populations associated with the Pacific Northwest ECOHAB Pseudonitzschia blooms. M.J. Wells, W. P. Cochlan and C.G. Trick. 2nd National Harmful Algal Bloom Symposium, Woods Hole Oceanographic Institution, December, 2003.

Ecological linkages between physical and oceanographic conditions and the seasonal growth and distribution of toxic algae blooms. Trainer, V.L., B.M. Hickey, and W.P. Cochlan. Western Juan de Fuca Ecosystem Symposium. Sydney BC, May, 2004.

Domoic Acid: The Synergy Of Iron, Copper And The Toxicity Of Diatoms. Wells, M. L. Trick, G.G., Cochlan, W.P. Hughes, M.P. and Trainer, V.L. ASLO Summer meeting, Santiago de Compostela, Spain, 2005

Determining the Effects of Fe(III) Complexing Ligands on Phytoplankton Community Structure Using a Sea-Going Continuous Culture Incubator. Pickell, L. D., Wells, M.L., Trick, C.G., Cochlan, W.P. and Herndon J. ASLO/AGU/TOS Ocean Sciences Meeting, February, 2006

Complexity of grow-out experiments: further iron stimulation of communities from iron fertilized mesoscale patch. Trick. C.G., Cochlan, W.P. and Wells, M.L. ASLO/AGU/TOS Ocean Sciences Meeting, February, 2006.

In addition, results have been presented at two workshops (Abstracts not published): PICES Iron Fertilization Panel Meeting (PICES-IFP) held in Victoria, B.C. (February 2004) and the ECOHAB-PNW Meeting held in Seattle, WA (May, 2004). Several papers are in various stages of preparation for submission in 2004/05, and one MSc. Thesis is nearing completion.

Wells, M.L., 2003. The level of iron enrichment required to initiate diatoms blooms in HNLC waters. *Marine Chemistry*, 82: 101-114.

Wells, M.L., Trick, C.G., Cochlan, W.P., Hughes, P. and Trainer, V.L., 2005. Domoic Acid: The synergy of iron, copper and the toxicity of diatoms. *Limnology and Oceanography*, 50(6): 1908-1917.

In addition, a minimum of 6 other manuscripts currently are in preparation for submittal in 2007 to detail the findings of the project.